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Structure of the chicken interferon-γ gene, and comparison to mammalian homologues

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Abstract

The sequence of the chicken interferon-γ (*ifn-γ*) gene was determined, one of the first non-mammalian cytokine gene structures to be elucidated. Initial genomic clones were amplified from chicken genomic DNA and were used to isolate a cosmid clone covering the entire gene for sequencing. The exon:intron structure of chicken *ifn-γ* is very similar to those of its mammalian homologues, with the exception of the third intron, which is markedly shorter in the chicken. The first exon contains both 5′ *UTR* and signal sequence and the first 22 aa of the mature protein. The remainder of the coding region lies in exons 2–4. Exon 4 also encodes the stop codon and the 3′ *UTR*, including two possible polyadenylation signals. A number of potential regulatory sequences similar to those found in mammals have been identified, in the promoter, in each intron and in the 3′ *UTR*. In the promoter, these include the TATAATA- and CCAT-boxes, a consensus GATA motif in the reverse orientation and a potential NF-κB binding site. Other regulatory elements identified in the promoters of mammalian *ifn-γ* genes are absent. Internal to the gene structure, regulatory sequences identified include elements found in the DNase I hypersensitivity region of the first intron of the human *ifn-γ* gene and several potential NF-κB binding sites. The 3′ *UTR* contains an AT-rich sequence, including nine repeats of the 'instability' motif ATTTA. As in mammals, chicken *ifn-γ* is a single copy gene. The gene is highly conserved, with no polymorphisms yet identified using either RFLP or SSCP in the coding region. However, promoter sequence polymorphisms between different inbred lines of chickens have been identified, with possible links to disease resistance. © 1998 Elsevier Science B.V.

Keywords: Cytokine; Avian; Nucleotide sequence; Genomic organisation

1. Introduction

Few homologues of mammalian cytokines have been cloned in the chicken — these include type I IFN [Sekellick et al., 1994 — also cloned in the duck (Schultz et al., 1995) and turkey (Suresh et al., 1995)], type II IFN (Digby and Lowenthal, 1995), IL-2 (Sundick and Gill-Dixon, 1997), IL-8 (Bedard et al., 1987; Sugano et al., 1987; Barker et al., 1993) and the TGF-β cytokine family (Jakowlew et al., 1988a,b,c, 1990). One gene for a cytokine receptor (type I IL-1R — Guida et al., 1992) has also been cloned. In addition, a chicken cytokine (myelomonocytic growth factor — Leutz et al., 1989; Sterneck et al., 1992) has been cloned for which no direct mammalian homologue has been identified. Only one of these genes has been studied at the genomic level (type I IFN, an intronless gene — Sick et al., 1996), and thus there is currently little information on the structure of avian cytokine genes, or regulatory features thereof.

IFN-γ has been implicated in the immune response to various avian diseases [for a review, see Kaiser (1996)]. Inbred lines of chickens differ in their resistance/susceptibility profiles to these diseases. Such profiles have been shown in mammals to be linked to polymorphisms in the promoters of cytokine genes (e.g. Pociot et al., 1991; McGuire et al., 1994; Walley and Cookson, 1996). Recently, links between gene polymorphisms and disease resistance have been demonstrated

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Abbreviations: aa, amino acid(s); Ab, antibody; bp, base pair(s); ConA, concanavalin A; DNase, deoxyribonuclease; GM-CSF, granulocyte macrophage colony stimulating factor; *ifn*, gene, cDNA or mRNA coding for IFN; IFN, interferon; IL, interleukin; nt, nucleotide(s); mAb, monoclonal Ab; MIP, macrophage inflammatory protein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PMA, phorbol 12-myristate 13-acetate; RFLP, restriction-fragment length polymorphism; RT-PCR, reverse transcription PCR; SDS, sodium dodecyl sulphate; SSC, 0.15 M NaCl/0.015 M Na_3.citrate pH 7.6; SSCP, single-stranded conformational polymorphism; TGF, transforming growth factor; *UTR*, untranslated region(s).

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for IFN-γ (Awata et al., 1994; Pravica et al., 1997). The first step in looking for such polymorphisms in avian *ifn-γ* was to clone the gene and analyse its organisation and putative regulatory sequences.

2. Materials and methods

2.1. Animals

Chickens of various inbred White Leghorn lines were produced and maintained at IAH, Compton, UK. Line 0, 6_1, 7_2, 15I and N birds originate from the Regional Poultry Research Laboratory, East Lansing, USA. Reaseheath C (C line) birds originate from the University of Cambridge, UK, P line birds from Cornell University, USA and Wellcome B14 line (W line) birds from Wellcome Research Laboratories, Beckenham, UK.

2.2. Genomic DNA cloning and analysis

Blood samples were collected in 3% sodium citrate. Cells were lysed by the addition of 1% saponin in PBS and erythrocyte nuclei isolated by centrifugation at $650 \times g$ for 5 min. Genomic DNA was isolated from these nuclei by a method based on that described by Sambrook et al. (1989). PCR techniques were used to generate genomic clones of chicken *ifn-γ*. Primer IFN1 (5′AGAAGACATAACTATTAGAA3′) corresponds to nucleotide (nt) positions −96 to −77 of the chicken *ifn-γ* cDNA clone isolated by Digby and Lowenthal (1995). Primer IFN4 (5′TTAGCAATTGCATCTCCTCT3′) is reverse complementary to nt positions 476–495. The resulting 3412 bp genomic fragments were cloned into a TA cloning vector, pTAg (R&D Systems). These clones were used to identify and isolate, by hybridisation, larger genomic clones from a commercial chicken cosmid library (adult Leghorn male liver DNA in pWE15, Clontech). The clones were sequenced using the PRISM™ Ready Reaction DyeDeoxy™ Terminator cycle sequencing kit (Applied Biosystems). The complete sequence of the clones was determined on each strand. Sequence data were analysed with the Wisconsin Package software (Genetics Computer Group; Devereux et al., 1984). The sequence has been deposited in the EMBL database with accession number Y07922.

RT-PCR experiments were carried out using the following protocol. First strand synthesis was for 2 h at 42°C in a 20 μl volume containing the reverse oligonucleotide primer (IFN16 — see Section 2.3) and Superscript II (Life Technologies). After denaturation of the polymerase at 94°C for 4 min, 10 μl of this reaction mix were added as DNA template to a 50 μl standard PCR reaction.

2.3. Mapping experiments

For RFLP analysis, DNA preparation and Southern blot analysis were carried out as described by Bumstead and Palyga (1992). Genomic DNA was prepared from each of the four parent birds of two Compton reference populations (line $6_1 \times$ line 7_2 and line 15I × line N), digested with restriction enzymes *Bam*HI, *Eco*RI, *Xba*I, *Rsa*I, *Taq*I, *Msp*I or *Hae*III and blotted on to nylon membrane (Hybond-N, Amersham). Hybridisation was carried out using DNA labelled with [^{32}P]dCTP (NEN) by nick translation (Rigby et al., 1977) at 42°C in 50% formamide overnight. Blots were washed twice at 55°C, and twice at 65°C, in 0.1 × SSC, 0.1% SDS.

For SSCP analysis, genomic DNA was prepared as described above. Amplification of 200–300-bp fragments across the length of the *ifn-γ* gene was carried out with 200 ng of chicken genomic DNA from each of the parent birds from the two mapping populations, 20 pmol of each primer (see below), and 2.5 U of *Taq* polymerase (Amersham) in 50 μl. Cycling conditions were 94°C for 1 min, 50°C for 2 min, 72°C for 2 min, for 30 cycles. Table 1 shows the primer pairs used in this study.

Table 1
Primer pairs used for SSCP analysis

Sense		Anti-sense	
Primer	nt	Primer	nt
IFN54	−2009 to −1988	IFN48	−1745 to −1765
IFN47	−1765 to −1745	IFN56	−1540 to −1560
IFN55	−1560 to −1540	IFN46	−1358 to −1376
IFN57	−1376 to −1358	IFN58	−1123 to −1142
IFN59	−1142 to −1123	IFN60	−894 to −914
IFN61	−914 to −894	IFN62	−643 to −663
IFN63	−663 to −643	IFN43	−334 to −354
IFN64	−354 to −334	IFN65	7 to −14
IFN1	22–41	IFN16	242–223
IFN9	223–242	IFN13	442–423
IFN12	423–442	IFN31	653–634
IFN32	634–653	IFN33	844–828
IFN34	828–844	IFN28	1047–1028
IFN27	1028–1047	IFN30	1266–1247
IFN29	1247–1266	IFN35	1490–1474
IFN36	1474–1490	IFN37	1690–1674
IFN38	1674–1690	IFN14	1870–1850
IFN15	1850–1870	IFN6	2094–2069
IFN5	2069–2094	IFN17	2280–2260
IFN18	2260–2280	IFN19	2459–2439
IFN20	2439–2459	IFN7	2657–2637
IFN8	2637–2657	IFN39	2850–2835
IFN40	2835–2850	IFN21	3043–3024
IFN22	3024–3043	IFN41	3243–3227
IFN42	3227–3243	IFN4	3433–3414
IFN69	3332–3352	IFN68	3698–3679
IFN67	3679–3698	IFN52	3991–3971
IFN53	3971–3991	IFN50	4271–4252

All nt numbers are calculated from the mRNA transcription start-point determined in this study.

Purified PCR products were run on polyacrylamide gels (0.5 × Sequagel MD, National Diagnostics) at 4 W for 6 h. Gels were then silver-stained using standard conditions (Sambrook et al., 1989).

3. Results and discussion

3.1. *Structure of the chicken* ifn-γ *gene*

The genomic structure of the gene encoding the chicken homologue of IFN-γ was determined. Primers designed from the published cDNA sequence (Digby and Lowenthal, 1995) were used to generate genomic clones by PCR with genomic DNA from line N chickens as template. Sequence analysis of these clones showed that the genomic structure of the chicken *ifn-γ* gene is remarkably similar to its mammalian homologues (Fig. 1, note numbering of nt from newly-determined transcriptional start-point) — the gene comprises four exons, each encoding similar numbers of aa to its mammalian equivalents, but only exon 2 has a significant sequence identity (72% in 69 nt for human — see Table 2). The first exon contains 5′ *UTR*, a signal sequence of 19 aa and the first 22 aa of the mature protein. Exons 2–4 encode 23, 60 and 40 aa, respectively. Exon 4 also encodes the stop codon and the 3′ *UTR*. Exons 2 and 4 are most highly conserved in aa sequence (56.5% and 42.5%, respectively — see Table 2). Of the three introns, the first two are longer than their mammalian equivalents; the third intron is, however, markedly shorter. As in mammalian genes, all three introns interrupt the open reading frame exactly between two codons (frame 0). The three introns also have the consensus sequence including 5′ GT and 3′ AG, but otherwise are dissimilar in sequence to mammalian *ifn-γ* introns (the best match is 62.1% identity in 58 nt with human intron 2 — see Table 2).

The G+C content of the chicken *ifn-γ* gene, in contrast to many other chicken genes (Riegert et al., 1996), is not significantly higher than its human homologue (see Table 2), either overall or in the wobble bases of the protein coding region. There is, however, a gradient from low G+C content at the 5′ end to high at the 3′ end, not present in human *ifn-γ*.

Interestingly, the gene shares certain possible internal regulatory regions with mammalian *ifn-γ* genes. The first intron contains several of the features described by Hardy et al. (1987) found in the DNase I hypersensitivity region of the first intron of the human *ifn-γ* gene. These include the sequence AGTTTCTTTG (nt 423–432), which has 90% nt identity with part of an *ifn-γ /IL-2/c-myc* consensus region (AGTNTCTTTT — Hardy et al., 1985) and a 30-base stretch of 80% uninterrupted Ts (nt 377–406). However, there is no obvious $(CACA)_n$ region, which is found in DNase I hypersensitivity sites

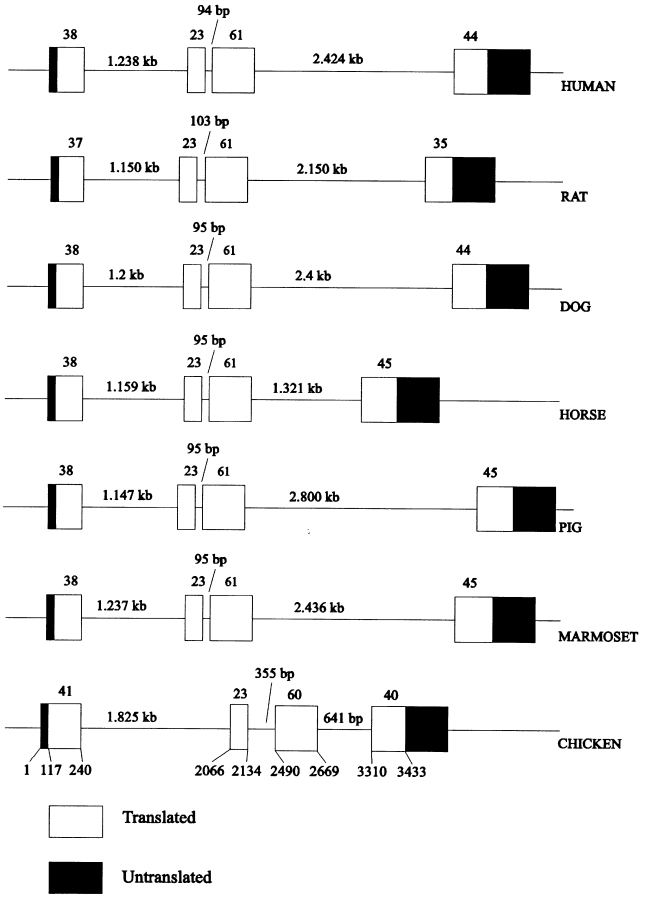


Fig. 1. Comparison of the gene structure of chicken *ifn-γ* with those of its mammalian homologues. The sizes of the first and third introns of the rat (Dijkema et al., 1985) and dog (Devos et al., 1992) are estimated. The other sequences were available in the databases—human (Accession Number V00536, Gray and Goeddel, 1982), horse (Accession Number A11777), pig (Accession Number X53085, Dijkmans et al., 1990) and marmoset (Accession Number X64659, Kaba et al., 1993). Numbers above the exons indicate numbers of aa encoded by each exon; those below the chicken exons indicate nt co-ordinates marking the exon:intron boundaries, calculated from the newly determined transcriptional start-point.

in the genes for human *ifn-γ*, human ϵ-globin and murine β-globin (Hardy et al., 1987), amongst others. This repeat is the site of one gene polymorphism implicated in disease susceptibility in man (Pociot et al., 1991). There are also three potential NF-κB family member binding sites (see Table 3), one in each intron, as described by Young (1996).

In order to isolate sequences peripheral to the coding sequences of the gene, the genomic clone generated by PCR was used to isolate cosmid clones from a chicken liver cosmid library (Clontech). Using these clones, the DNA sequence approximately 2 kb upstream of the *ifn-γ* gene was determined. This region presumably contains most of the *ifn-γ* promoter. In mammals, many of the important transcriptional regulatory elements for the *ifn-γ* gene lie within the first 150 bp upstream of the

Table 2
Comparison of the gene structure of chicken and human *ifn-γ* genes

	Length (nt)[a]	Percentage G+C[a]	Identity (nt)[b]	Percentage identity (aa)
5′ *UTR*	117 (128)	39 (38)	64.8% in 54 bp	
Exon 1	123 (114)	34 (36)	46.3%	19.5
Intron 1	1825 (1239)	39 (31)	54.2% in 201 bp	
Exon 2	69 (69)	42 (38)	72%	56.5
Intron 2	355 (95)	46 (39)	62.1% in 58 bp	
Exon 3	180 (183)	43 (35)	45.4%	21.7
Intron 3	642 (2425)	52 (39)	57.1% in 84 bp	
Exon 4	123 (135)	47 (47)	45.9%	42.5
3′ *UTR*	~763[c] (585)	32 (33)	63.8% in 149 bp	

[a]Values in parentheses are for the corresponding regions of the human *ifn-γ* gene.
[b]Values for nt identity of non-translated regions of the gene are the best matches as generated by FASTA analysis.
[c]The actual 3′ end of the chicken gene is yet to be determined.

transcriptional start-point. Fig. 2 shows a comparison of these nucleotide sequences for several mammalian *ifn-γ* genes and chicken *ifn-γ*. The putative transcriptional elements in the promoter, initially identified in other mammalian species by their homology with human and murine *ifn-γ* gene elements, are underlined. Some of these elements are conserved in the chicken. These include the TATAATA- and the CCAT-boxes. The former is 100% conserved in the chicken (Fig. 2, element E), whilst the CCAT-box has 5/6 nucleotide identity in its core sequence (CCATCT — Fig. 2, element B). The promoter also contains a potential NF-κB family member binding site (see Table 3).

Penix et al. (1993) identified two essential regulatory elements in the human *ifn-γ* promoter that are also conserved in other mammalian *ifn-γ* gene promoters. The distal conserved element contains a consensus GATA motif in the reverse orientation (Fig. 2, element A) and a potential regulatory motif, found in the promoter regions of the GM-CSF and MIP genes that includes the CCAT-box (Fig. 2, element B). Both sequences are conserved in the chicken *ifn-γ* promoter. The GATA motif shows 5/6 nt identity with its mammalian homologue, and the GM-CSF/MIP motif shows 7/10 nt identity. The proximal conserved element (Fig. 2, element C) shares homology with the NFIL-2A element in the *il-2* promoter, which, in turn, has homology at

Table 3
Potential NF-κB family member binding sites

Sequence[a]	Location
GGGAMTNYCC	Consensus sequence
GGGATTCCCT	Promoter, nt −231 to −222
CTGATTTTTC	Intron 1, nt 979–988
TTCATTTTCC	Intron 2, nt 2431–2440
CACACTTTCC	Intron 3 (r)[b], nt 2802–2793

[a]Underlined bases indicate homology to consensus sequence.
[b](r), reverse orientation.

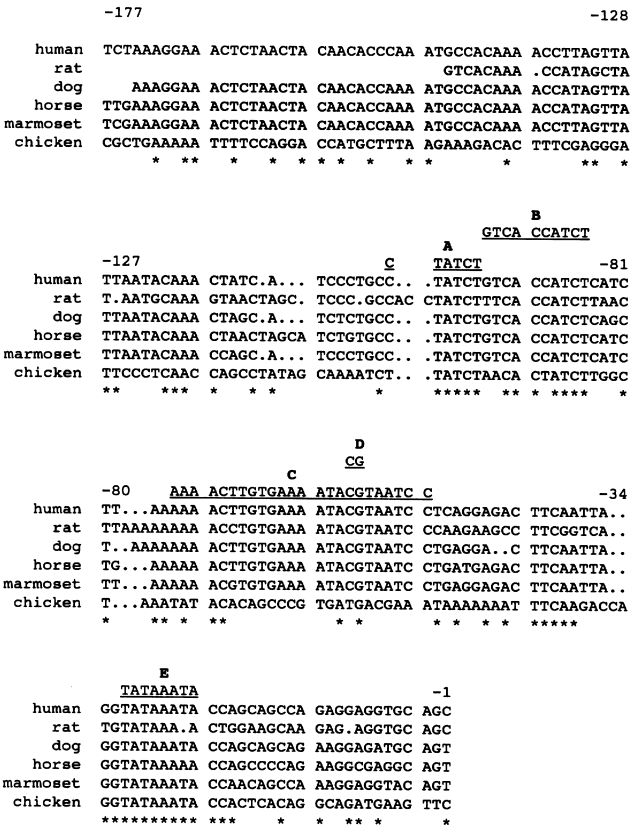


Fig. 2. Comparison of the promoter sequence of chicken *ifn-γ* with those of its mammalian homologues. Promoter sequences known to be involved in *ifn-γ* expression in mammalian genes are shown underlined above the pile-ups. Element A indicates a consensus GATA motif in the reverse orientation. Element B includes the CCAT-box and is a potential regulatory motif also found in the promoter regions of mammalian GM-CSF and MIP genes. Element C is a proximal conserved element that shares homology with the NFIL-2A element in the *il-2* promoter, which in turn has homology at its 3′ end with the consensus octamer site (ATGCAAAT). Element D is a conserved CpG target site for methylation. Element E is the TATAATA-box. Asterisks indicate nt conserved between the chicken and human promoters.

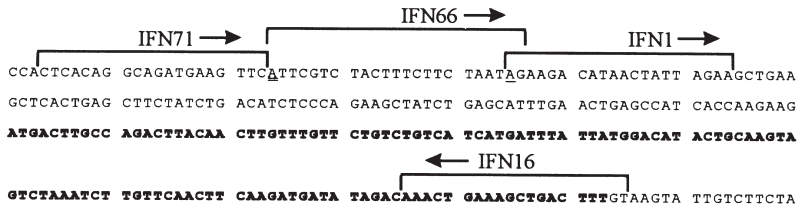
its 3′ end with the consensus octamer site (ATGCAAAT). This regulatory element is apparently absent in the chicken *ifn-γ* promoter (5/24 nt identity), suggesting that the former, and its associated regulatory mechanisms, may have arisen after the divergence of avians and mammals.

In mammals, methylation at a conserved CpG target site (Fig. 2, element D) in the promoter has been shown to have a role in regulating *ifn-γ* gene expression [for a review, see Young (1996)]. This site is absent in the chicken *ifn-γ* promoter, and therefore, methylation presumably does not play such an important role in the expression of chicken IFN-γ.

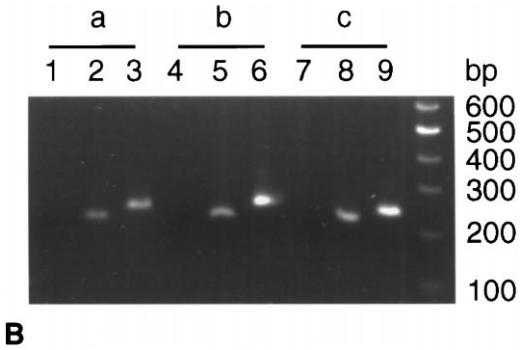
The sequence of the *ifn-γ* cDNA clone isolated by Digby and Lowenthal (1995) extends 96 nt upstream of the translational start-point. Analysis of the sequence of the *ifn-γ* genomic clone suggested that this reported transcriptional start-point was incorrect, and that the true start-point lay an additional 21 nt upstream. To test this hypothesis, RT-PCR experiments were carried out using primers recognising sequences upstream of the putative transcriptional start-point (IFN71), beginning at the putative transcriptional start-point (IFN66) and beginning at the reported transcriptional start-point (IFN1) (see Fig. 3A), and an anti-sense primer at the end of the first exon (IFN16). Fig. 3B shows the RT-PCR products generated using these primer combinations. Products of 221 bp were generated with primers IFN1 and IFN16, as expected. However, products of the predicted size (242 bp) were generated with primers IFN66 and IFN16, but none with IFN71 and IFN16, indicating that the true transcriptional start-point may indeed be that suggested from the genomic sequence. This hypothesis is supported by the fact that this start-point is 31 nt downstream of the TATA-box, and that it is very close (8/9 nt identity) to the consensus Py_2CAPy_5 transcriptional initiator sequence (the first base of the putative transcription product is double-underlined).

Weining et al. (1996) independently cloned a cDNA for *ifn-γ* (Genbank Accession Number X99774). Their 5′*UTR* was identical to the published *ifn-γ* cDNA (Digby and Lowenthal, 1995), but with an additional 9 nt at the 5′ end. There was no homology between these extra 9 nt and the equivalent sequence in our genomic clones.

There are two potential polyadenylation signals (AATAAA) 715–730 nt 3′ of the translational stop codon (nt 4145–4150 and nt 4157–4162; see Fig. 4).



A



B

Fig. 3. Attempts to determine the transcriptional start-point of chicken *ifn-γ*. (**A**) 5′ end of the chicken *ifn-γ* gene, showing locations of primers used in RT-PCR experiments. IFN71 lies upstream of the putative transcriptional start-point (double-underlined); IFN66 begins at this putative transcriptional start-point and extends to two nucleotides downstream of the published transcriptional start-point (underlined); IFN1 begins at the published transcriptional start-point. IFN16, the anti-sense primer, begins at the junction between intron 1 and exon 1 (shown in bold) and extends into exon 1. (**B**) 1.5% TBE agarose gel showing RT-PCR products from mRNA extracted from splenocytes cultured in the presence of (a) a mAb (AV33) against the common α-chain of the chicken TCR-αβ for 18 h, (b) ConA for 24 h and (c) PMA for 24 h. First-strand synthesis was for 2 h at 42°C in the presence of Superscript II (Life Technologies) and primer IFN16. Amplification was carried out with 10 μl of the first-strand synthesis reaction, 20 pmol of each primer (see below), and 2.5 U of *Taq* polymerase (Amersham) in 50 μl. Cycling conditions were 94°C for 1 min, 50°C for 2 min, 72°C for 2 min, for 30 cycles. Lanes 1, 4 and 7, primers IFN71 and IFN16; lanes 2, 5 and 8, primers IFN1 and IFN16; lanes 3, 6 and 9, primers IFN66 and IFN16.

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            M  N  D  L  R  I  Q  R  K  A  A  N  E  L  F  S  I
CTCAAAACAG ATGAACGACT TGAGAATCCA GCGCAAAGCC GCGAATGAAC TCTTCAGCAT   3360
  L  Q  K  L  V  D  P  P  S  F  K  R  K  R  S  Q  S  Q  R  R
CTTACAGAAG CTGGTGGATC CTCCGAGTTT CAAAAGGAAA AGGAGCCAGT CTCAGAGGAG   3420
  C  N  C  *
ATGCAATTGC TAATGGCGTC TTATGACCTC CTGTGCTCAA CTATTTTAAA TTTTACAATG   3480
CACAATTTTT ATGTTTTGAT TTTTTAACTG AGTTTTTATA CATTTATTTA TTAATATTTA   3540
AGTATTTTAA ATAATTATTT ATATATAAAA AAAAAAACAG GCAAACAATG GAAGTATTTA   3600
TACCTCCTAC TGCTGTGTAA GAAACGATTT TGTCTTAAAA TACTGTCTAT CTGTTGTATG   3660
TTTGTTGACC TGAAAATACC GAATGAGGTG ATGTTTACCG AGTTTCTGTG TGGAAATACT   3720
GAATTGACGT TGATACTGTA CTCAGGAAAA CCCACTCATA CCTGCTCAGC TCTAAGCATA   3780
TCTAAATCCA AATCAAGGAA GTAGACTTGC TTTAAGGTGA GAAAATGCTG AAGCACTTTT   3840
CTGAACTGGG ATCTGAGAGA TTTATTACTG ATAGTTATTG TTATGCACTG AAGCAACTGA   3900
GAGGCCAGGC TACTTGGCAG CTCCAGGAAA TGTGACACTC TATTGCACTG ACTTAACTTC   3960
AACTCATTTA CTATGAACGT CTCTGTACTT CTTTGTATTG AATCATCTAA GTGTGTCTGA   4020
CATCAGTTTA TTTATTTGGA AGTAGCAGTA TGGAAGATTT TATCTTAAGG ACTGTATTTT   4080
TGTACTTGAA TATTATTTAA AACTTTGGAT TTTACAATGA AGGTTTCTTA AAATTTGGAT   4140
ATGAAATAAA GAAAAGAATA AATTAATTAA GTGTGCTTGC ACATTTATTC ACCAGAATCT   4200
CTGTGAAAAG CAGATTTTCA GTTTTGCTTG ATGTTGTCAC AAAAGA                  4246
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Fig. 4. Nt sequence of the 3′-*UTR* of the chicken *ifn-γ* gene. The two potential polyadenylation signals are double-underlined and the 'instability' motifs (ATTTA) overlined.

There is no obvious polyadenylation signal downstream of the stop codon in the original published *ifn-γ* cDNA sequence (Digby and Lowenthal, 1995). The first 300 nt of the original 3′ sequence are very similar to those of the two cosmid clones sequenced in this study, but after this, there is little homology. In contrast, the 3′ *UTR* described by Weining et al. (1996) is virtually identical to the 3′ *UTR* from this study (5/734 nt differences).

The 3′ *UTR* contains nine repeats of the 'instability' motif ATTTA (see Fig. 4). This element, present in mammalian cytokines and proto-oncogenes (Shaw and Kamen, 1986), mediates rapid mRNA degradation and is a recognition site for a RNase E-like activity that cleaves RNA sequences in man (Wennborg et al., 1995).

3.2. Mapping experiments

RFLP analysis was carried out for both the line $6_1 \times$ line 7_2, and the line N × line 15I Compton mapping populations using *Bam*HI, *Eco*RI, *Xba*I, *Rsa*I, *Taq*I, *Msp*I and *Hae*III. Hybridisation was carried out using the initial genomic clone generated by PCR labelled with [^{32}P]dCTP as a probe. No RFLPs were identified with this panel of restriction enzymes. Further hybridisation experiments were carried out using other restriction enzymes, but again no RFLPs were identified (data not shown). These experiments did, however, show that chicken *ifn-γ* is a single copy gene, as are its mammalian homologues, as hybridisation only occurred to bands of sizes predicted from the sequence of the genomic clones.

SSCP analysis was carried out on 200–300-bp PCR fragments covering the majority of the gene (i.e. from nt −2009 to 4271: see Table 2 for details). No polymorphisms were detected for any PCR fragment in either of the mapping populations. This indicates a remarkable degree of conservation for IFN-γ between different lines of chickens, and contrasts with other chicken genes that have been shown to be highly polymorphic, in some cases more so than their mammalian counterparts (Tregaskes et al., 1996).

3.3. Comparison of ifn-γ promoter sequences from different inbred lines of chickens

The *ifn-γ* promoter region was cloned from genomic DNA from eight inbred lines of chickens (lines 0, 6_1, 7_2, 15I and N, and C line, P line and W line) by PCR using primers IFN63 and IFN65. The resulting 670 nucleotide product was sequenced for each line. The promoter sequences fell into two groups. One group (lines 0 and 15I and C line) was identical in sequence to the originally cloned line N sequence. The other lines all fell into a second group with two base pair changes, both C for T, at bases −112 and −317. Unfortunately, the parent birds for each of the mapping populations available at IAH both lie in the same group (lines N and 15I in one group, lines 6_1 and 7_2 in the other), and therefore, these promoter polymorphisms cannot be used to map the gene. These differences also agree with the absence of identifiable SSCP-PCR polymorphisms in the promoter. Neither of these two nucleotide changes lies in any of the regulatory regions identified by sequence analysis and comparison to known mammalian *ifn-γ* promoters. However, this does not rule out their playing a role in the expression of IFN-γ in the chicken.

Recently, sequence polymorphisms in the promoter of mammalian *ifn-γ* genes (Awata et al., 1994; Pravica et al., 1997) have been shown to play a role in resistance to disease. The relative disease resistance/susceptibility profiles of the inbred lines of chickens at IAH are well established (Bumstead et al., 1991), so the correlation between disease resistance and *ifn-γ* promoter sequence was investigated. There is no obvious relationship between *ifn-γ* promoter polymorphisms and known disease resistance profiles for commercially important avian diseases, whether protozoan (coccidiosis), bacterial (salmonellosis) or viral (Marek's disease, infectious bronchitis or avian leukosis). However, there is some degree of correlation between resistance to *Escherichia coli* infection and promoter sequence, in that lines 6_1 and 7_2 are susceptible, whereas C line, line N and line 15I are, to some degree, resistant (Bumstead et al., 1991). However, this profile needs to be extended to include the other lines sequenced in this study (P and W lines, and line 0) to confirm any such correlation.

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References

Awata, T., Matsumoto, C., Urakami, T., Hagura, R., Amemiya, S., Kanazawa, Y., 1994. Association of polymorphism in the interferon γ gene with IDDM. Diabetologia 37, 1159–1162.

Barker, K.A., Hampe, A., Stoeckle, M.Y., Hanafusa, H., 1993. Transformation-associated cytokine 9E3/CEF4 is chemotactic for chicken peripheral blood mononuclear cells. J. Virol. 67, 3528–3533.

Bedard, P.-A., Alcorta, D., Simmons, D.L., Luk, K.-C., Erikson, R.L., 1987. Constitutive expression of a gene encoding a polypeptide homologous to biologically active human platelet protein in Rous sarcoma virus-transformed fibroblasts. Proc. Natl. Acad. Sci. USA 84, 6715–6719.

Bumstead, N., Millard, B.J., Barrow, P., Cook, J.K.A., 1991. Genetic basis of disease resistance in chickens. In: Owen, J.B., Axford, R.F.E. (Eds.), Breeding for Disease Resistance in Farm Animals. CAB International, Wallingford, UK, pp. 10–23.

Bumstead, N., Palyga, J., 1992. A preliminary linkage map of the chicken genome. Genomics 13, 690–697.

Devereux, J., Haeberli, P., Smithies, O., 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12, 387–395.

Devos, K., Duerinck, F., van Audenhove, K., Fiers, W., 1992. Cloning and expression of the canine interferon-γ gene. J. Interferon Res. 12, 95–102.

Digby, M.R., Lowenthal, J.W., 1995. Cloning and expression of the chicken interferon-γ gene. J. Interferon Cyt. Res. 15, 939–945.

Dijkema, R., van der Meide, P.H., Pouwels, P.H., Caspers, M., Dubbeld, M., Schellekens, H., 1985. Cloning and expression of the chromosomal immune interferon gene of the rat. EMBO J. 4, 761–767.

Dijkmans, R., Vandenbroeck, K., Beuken, E., Billiau, A., 1990. Sequence of the porcine interferon-gamma gene. Nucleic Acids Res. 18, 4259

Gray, P.W., Goeddel, D.V., 1982. Structure of the human immune interferon gene. Nature 298, 859–863.

Guida, S., Heguy, A., Melli, M., 1992. The chicken IL-1 receptor: differential evolution of the cytoplasmic and extracellular domains. Gene 111, 239–243.

Hardy, K.J., Peterlin, B.M., Atchison, R.E., Stobo, J.D., 1985. Regulation of expression of the human interferon γ gene. Proc. Natl. Acad. Sci. USA 82, 8173–8177.

Hardy, K.J., Manger, B., Newton, M., Stobo, J.D., 1987. Molecular events involved in regulating human interferon-γ gene expression during T cell activation. J. Immunol. 138, 2353–2358.

Jakowlew, S.B., Dillard, P.J., Sporn, M.B., Roberts, A.B., 1988a. Nucleotide sequence of chicken transforming growth factor-beta 1 (TGF-β 1). Nucleic Acids Res. 16, 8730.

Jakowlew, S.B., Dillard, P.J., Kondaiah, P., Sporn, M.B., Roberts, A.B., 1988b. Complementary deoxyribonucleic acid cloning of a novel transforming growth factor-β messenger ribonucleic acid from chick embryo chondrocytes. Mol. Endocrinol. 2, 747–755.

Jakowlew, S.B., Dillard, P.J., Sporn, M.B., Roberts, A.B., 1988c. Complementary deoxyribonucleic acid cloning of a messenger ribonucleic acid encoding transforming growth factor β4 from chicken embryo chondrocytes. Mol. Endocrinol. 2, 1186–1195.

Jakowlew, S.B., Dillard, P.J., Sporn, M.B., Roberts, A.B., 1990. Complementary deoxyribonucleic acid cloning of an mRNA encoding transforming growth factor-β2 from chicken embryo chondrocytes. Growth Factors 2, 123–133.

Kaba, A., Eladari, M.-E., Mohammed-Ali, K., Rhodes-Feuillette, A., Galibert, F., 1993. The molecular cloning and sequence of the common marmoset interferon-gamma (MaIFN-γ) gene. DNA Seq. J. DNA Seq. Mapping 3, 387–392.

Kaiser, P., 1996. Avian cytokines. In: Davison, T.F., Morris, T.R., Payne, L.N. (Eds), Poultry Immunology. Carfax, Abingdon, UK, pp. 83–114.

Leutz, A., Damm, K., Sterneck, E., Kowenz, E., Ness, S., Frank, R., Gausepohl, H., Pan, Y.-C.E., Smart, J., Hayman, M., Graf, T., 1989. Molecular cloning of the chicken myelomonocytic growth factor (cMGF) reveals relationship to interleukin 6 and granulocyte colony stimulating factor. EMBO J. 8, 175–181.

McGuire, W., Hill, A.V.S., Allsopp, C.E.M., Greenwood, B.M., Kwiatkowski, D., 1994. Variation in the TNF-α promoter region associated with susceptibility to cerebral malaria. Nature 371, 508–511.

Penix, L., Weaver, W.M., Pang, Y., Young, H.A., Wilson, C.B., 1993. Two essential regulatory elements in the human interferon γ promoter confer activation specific expression in T cells. J. Exp. Med. 178, 1483–1496.

Pociot, F., Molvig, J., Wogensen, L., Worsaae, H., Dalboge, H., Baek, L., Nerup, J., 1991. A tumour necrosis factor beta gene polymorphism in relation to monokine secretion and insulin-dependent diabetes mellitus. J. Scand. Immunol. 33, 37–49.

Pravica, V.P., Borreiro, L.F., Hutchinson, I.V., 1997. Genetic regulation of interferon-γ production. Biochem. Soc. Trans. 25, 176S

Riegert, P., Anderson, R., Bumstead, N., Dohring, C., Dominguez-Steglich, M., Engberg, J., Salomonsen, J., Schmid, M., Schwager, J., Skjodt, K., Kaufman, J., 1996. The chicken β2-microglobulin gene is located on a non-major histocompatability complex microchromosome: a small, G + C-rich gene with X and Y boxes in the promoter. Proc. Natl. Acad. Sci. USA 93, 1243–1248.

Rigby, D.W.J., Dieckmann, M., Rhodes, C., Berg, P., 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. 113, 237–251.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schultz, U., Kock, J., Schlicht, H.-J., Staeheli, P., 1995. Recombinant duck interferon: a new reagent for studying the mode of interferon action against hepatitis B virus. Virology 212, 641–649.

Sekellick, M.J., Ferrandino, A.F., Hopkins, D.A., Marcus, P.I., 1994. Chicken interferon gene: cloning, expression and analysis. J. Interferon Res. 14, 71–79.

Shaw, G., Kamen, R.A., 1986. Conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46, 659–667.

Sick, C., Schultz, U., Staeheli, P., 1996. A family of genes coding for two serologically distinct chicken interferons. J. Biol. Chem. 271, 7635–7639.

Sterneck, E., Blattner, C., Graf, T., Leutz, A., 1992. Structure of the chicken myelomonocytic growth factor gene and specific activation of its promoter in avian myelomonocytic cells by protein kinases. Mol. Cell. Biol. 12, 1728–1735.

Sugano, S., Stoeckle, M.Y., Hanafusa, H., 1987. Transformation by Rous sarcoma virus induces a novel gene with homology to a mitogenic platelet protein. Cell 49, 321–328.

Sundick, R.S., Gill-Dixon, C., 1997. A cloned chicken lymphokine homologous to both mammalian IL-2 and IL-15. J. Immunol. 159, 720–725.

Suresh, M., Karaca, K., Foster, D., Sharma, J.M., 1995. Molecular and functional characterisation of turkey interferon. J. Virol. 12, 8159–8163.

Tregaskes, C.A., Bumstead, N., Davison, T.F., Young, J.R., 1996. Chicken B-cell marker chB6 (Bu-1) is a highly glycosylated protein of novel structure. Immunogenetics 44, 212–217.

Walley, A.J., Cookson, W.O.C.M., 1996. Investigation of an

interleukin-4 promoter polymorphism for associations with asthma and atopy. J. Med. Genet. 33, 689–692.
Weining, K.C., Schultz, U., Munster, U., Kaspers, B., Staeheli, P., 1996. Biological properties of recombinant chicken interferon-γ. Eur. J. Immunol. 26, 2440–2447.
Wennborg, A., Sohlberg, B., Angerer, D., Klein, G., von Gabain, A., 1995. A human RNase E-like activity that cleaves RNA sequences involved in mRNA stability control. Proc. Natl. Acad. Sci. USA 92, 7322–7326.
Young, H.A., 1996. Regulation of interferon-γ gene expression. J. Interferon Cyt. Res. 16, 563–568.